

Influence of Temperature and pH on the Solubility of α_{s1} -, β - and κ -Casein

Abstract

Both temperature and pH affect the solubility of κ -casein and β -casein in the region of their isoelectric points. This investigation covered a range of from 5 to 25 C and of from 3.9 to 5.2 pH. From pH 4.7 to pH 5.2, κ -casein became more soluble as the temperature was decreased. However, temperature had no effect on the solubility of κ -casein between pH 3.9 and 4.6. The solubility of β -casein increased as the temperature decreased over the entire range of pH 3.9 to 5.2. The increased solubility of β -casein was greatest between 5 and 15 C. Solubility of α_{s1} -casein was affected by temperature to a minor extent; α_{s1} -casein solubility was

slightly greater at 5 C than at 25 C at pH values below 4.0 and above 5.0.

During some studies on κ -casein it was observed that this protein was soluble in acetate buffer at pH 5.1 at 5 C. When the solution was warmed to room temperature, the κ -casein precipitated. Warner (11) observed that β -casein behaved similarly. He found that this protein is soluble at 2 C and pH 4.9, but precipitates upon warming to room temperature. α -Casein at pH 4.9, however, is insoluble at 2 C and 25 C. These observations suggested that it would be of interest to compare the solubility of κ -casein to that of β -casein, near the isoelectric point of both proteins. The behavior of α_{s1} -casein (obtained from the α -

casein complex) was also studied for comparison.

Experimental Procedure

κ -Casein B was isolated from acid-precipitated whole casein by the method of Zittle and Custer (12). β -Casein A¹ was isolated by the method of Aschaffenburg (1) and further purified by DEAE-cellulose chromatography (9). α_{s1} -Casein was isolated from pooled milk by the method of Zittle and Custer (12).

Protein was determined from absorbance at 290 $m\mu$ in a Beckman DU Spectrophotometer,¹ with a 1 cm cell. To clarify the solution, a drop of 50% sodium hydroxide was added to each 10 ml sample before reading the absorbance. Maximum absorption occurs at 290 $m\mu$ rather than 280 $m\mu$ when the pH is higher than 11.0 due to the ionization of tyrosine. Protein was calculated from the extinction coefficient 12.2 for κ -casein (12), 4.6 for β -casein (10), and 10.2 for α_{s1} -casein (12). As these extinction coefficients were determined at 280 $m\mu$, corrections were made for the higher absorption at 290 $m\mu$.

Buffer solutions were mixtures of 0.2 M sodium acetate and 0.2 M acetic acid from pH 3.9 to 5.2, ionic strength varied from .024 to .165. pH values were determined on the Beckman expanded scale pH meter standardized at 5 C with a pH 4.0 buffer. pH of the protein solutions was measured at 5 C to avoid precipitation of the protein.

Solubilities of κ -casein and β -casein were determined at 5, 10, 15, 20, and 25 C and at pH values of 3.9, 4.0, 4.13, 4.3, 4.6, 4.7, 4.8, 5.0 and 5.2. Stock protein solutions, containing approximately 1 g casein (α_{s1} , β or κ)/100 ml water, were adjusted to pH 6.7 with N sodium hydroxide. Casein solutions and buffers were equilibrated in a water bath at the specified temperature for 15 min. After equilibration, 2 ml casein solution were added to 2 ml acetate buffer at the indicated pH's. The solutions were equilibrated in the water bath for 30 min and were then centrifuged at $9,000 \times g$ for 10 min in a Beckman Model L4 ultracentrifuge with a Type 50 titanium rotor. Temperature of the centrifuge rotor and chamber was maintained to within 1 C of the selected temperature during the run. The rotor was equilibrated at the appropriate temperature in a water bath prior to centrifugation. The supernatant was decanted and stored at 5 C. Protein and

pH were then determined on the supernatant solution. Similar experiments were with α_{s1} -casein at two temperatures, 5 and 25 C.

Results and Discussion

Figure 1 shows the effect of temperature and pH on the solubility of β -casein. Over the pH range tested (pH 3.9 to 5.2) there was a marked effect of temperature on solubility; the solubility of β -casein increased as the temperature was lowered.

Figure 2 shows the results of similar experiments with κ -casein. Unlike the results with β -casein, temperature has little effect on the solubility of κ -casein between pH 3.9 and 4.6. From pH 4.7 to 5.2, however, κ -casein, like β -casein, became more soluble as the temperature was decreased.

For comparison, Figure 3 shows the effect of temperature on α_{s1} -casein over the same pH range at 5 and 25 C. α_{s1} -Casein is considerably less soluble than β -casein and κ -casein. This protein, like β -casein, is more soluble at 5 than at 25 C, but the effect of temperature on solubility is minimal.

A distinguishing characteristic of β -casein is that its solubility in the region of its isoelectric point increases as its solution temperature is lowered. This effect is most pronounced between 5 and 15 C. Although no conclusions can be reached concerning the degree of polymerization of the soluble β -casein in these experiments, it is of interest that other workers have observed changes in the β -casein structure in this temperature range. Sullivan et al. (8), for example, studied the effect of temperature on the physical characteristics of α - and β -caseins in veronal and phosphate buffers (pH 7.8 and 6.2). By sedimentation analyses

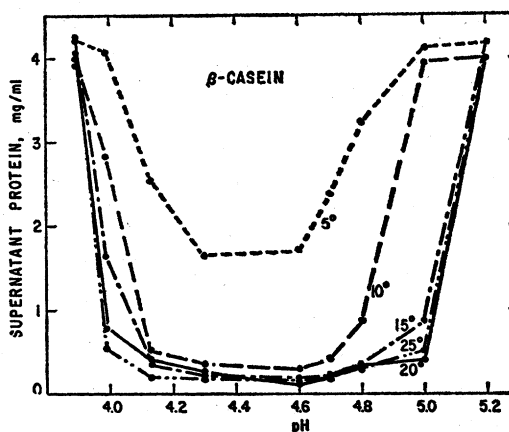


FIG. 1. Solubility of β -casein at various temperatures and pH values.

¹ Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture, to the exclusion of others that may be suitable.

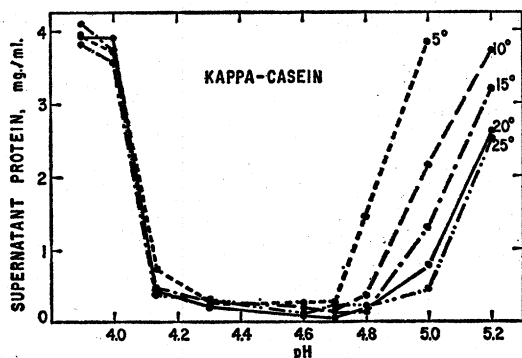


FIG. 2. Solubility of κ -casein at various temperatures and pH values.

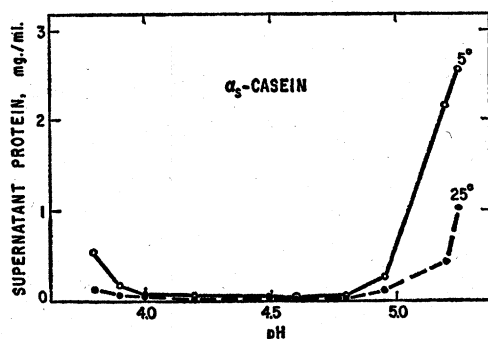


FIG. 3. Solubility of α_{s1} -casein at various temperatures and pH values.

and viscosity measurements they demonstrated that β -casein forms aggregates at room temperature, but is monomeric at 0 C. This unique casein begins to dissociate at 15 C. Our results on β -casein (Fig. 1) corroborate those of Sullivan. The earlier results of Sullivan have been confirmed recently by Noelken and Reibstein (5) and Payens and van Markwijk (6).

Ho and Chen (3) produced evidence that the aggregation of α_{s1} -casein is dependent upon ionic strength rather than temperature at a neutral pH. By osmotic pressure measurements at pH 7 they showed that the molecular weight of α_{s1} -casein shows little change from 4 to 20 C. However, α_{s1} -casein goes from a monomer in 0.01 M KCl to a trimer in 0.1 M KCl. Our results suggest that α_{s1} -casein is less affected by temperature than β -casein or κ -casein.

It is of interest that κ -casein also increases in solubility as the temperature is lowered, but only in the restricted range of pH 4.7 to 5.2. Further experiments will be necessary to determine whether this effect can be attributed to a decrease in size of the κ -casein aggregate

and whether ionic strength or the nature of the buffer has an effect.

It is well known that caseins, like many other proteins, have minimum solubility at their isoelectric points. The isoelectric points of α_{s1} -casein, β -casein and κ -casein are 4.1, 4.5 and 4.1 (7). Caseins, by definition, are phosphoproteins which precipitate from skim milk at pH 4.6 and 20 C. These experiments show that the precipitation of α_{s1} -casein, β -casein, and κ -casein, near their isoelectric points, is affected by temperature.

Although no completely satisfactory explanation for the effect of temperature on the aggregation of casein has been advanced, intermolecular hydrophobic interactions are probably involved. Kauzmann (4) has pointed out that hydrophobic interactions involving aliphatic side-chains are more stable at room temperature than at 0 C. Hill and Wake (2), using Bigelow's scale, rank the caseins in order of decreasing hydrophobicity $\beta > \alpha_{s1} > \kappa$. This agrees with the work here since β -casein with the largest average hydrophobicity shows the largest effect of temperature on solubility. Although the average hydrophobicity of κ -casein is less than that of α_{s1} -casein, κ -casein shows more temperature dependence. This apparent anomaly might be related to the high concentration of hydrophobic residues in the N-terminal portion of the κ -casein molecule (2). That κ -casein and α_{s1} -casein show the temperature effect only in a limited pH range suggests that factors other than hydrophobic interactions are involved.

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